

Applicants : Alexander Gad and Dora Lis  
Serial No. : Not Yet Known(Continuation of U.S. Serial  
No. 09/816,989, filed March 32, 2001)  
Filed : Herewith  
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**In the Specification**

On page 1, after the title please replace the paragraph which begins on line 5 and ends on line 6 with the following new paragraph:

The present application is a continuation of U.S. Serial No. 09/816,989, filed March 23, 2001, which is a continuation of PCT International Application No. PCT/US99/22402, filed September 24, 1999, which claims the benefit of provisional application U.S. Provisional Application Nos. 60/101,825 and 60/101,693, both filed September 25, 1998, which ~~is~~ are incorporated by reference herein.

On Page 3, replace the paragraph that begins on line 13 and ends on line 27 with the following paragraph:

Glatiramer acetate (Copolymer 1; Cop 1; hereinafter GLAT copolymer) is a mixture of polypeptides composed of alanine, glutamic acid, lysine, and tyrosine in a molar ratio of approximately 4.6:1.5:3.6:1.0, respectively, which is synthesized by chemically polymerizing the four amino acids, forming products with average molecular weights ranging from about 4000 to about 13,000 daltons. The corresponding molar fractions are approximately 0.427 for alanine, 0.141 for glutamic acid, 0.337 for lysine and 0.093 for tyrosine, and may vary by about +/-10%. Related copolymers are mixtures of polypeptides composed of three (thus, "terpolymers") of the four aforementioned amino acids. Copolymer 1 and the terpolymers address the innate heterogeneity of the mammalian immune system and human population and are effective for treatment of autoimmune diseases and other

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immune conditions. Preferred average molecular weight ranges and processes of making terpolymers are described in U.S. Pat. No. 5,800,808, which is hereby incorporated by reference in its entirety. Copolymer-1, according to the present invention, may be prepared by methods known in the art, for example, the process disclosed in U.S. Pat. No. 3,849,550, wherein the N-carboxyanhydrides of tyrosine, alanine,  $\gamma$ -benzyl glutamate and E-N-trifluoro-acetyllysine are polymerised at ambient temperature in anhydrous dioxane with diethylamine as initiator. The deblocking of the  $\gamma$ -carboxyl group of the glutamic acid is effected by hydrogen bromide in glacial acetic acid and is followed by the removal of the trifluoroacetyl groups from the lysine residues by 1M piperidine. For the purposes of the application, the terms "ambient temperature" and "room temperature" should be understood to mean a temperature ranging from about 20° to about 26° C. The copolymer-1 with the required molecular weight profile can be obtained either by methods known per se. Such methods include chromatography of copolymer-1 containing high molecular weight species and collecting the fractions without the undesired species or by partial acid or enzymatic hydrolysis to remove the high molecular weight species with subsequent purification by dialysis or ultrafiltration. A further method to obtain copolymer-1 with the desired molecular weight profile is by preparing the desired species while the amino acids are still protected and then obtain the correct species directly upon removing the protection. The compositions of the present invention may be formulated by conventional methods known in the art. Preferably, the composition is lyophilized and formed into an aqueous solution suitable for subcutaneous injection. Alternatively, copolymer-1 may be

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formulated in any of the forms known in the art for preparing oral, nasal, buccal, or rectal formulations of peptide drugs. Also contemplated by the invention are other copolymers comprised of other combinations of three, four, or five or more amino acids.

Also replace the paragraph that begins on page 3, line 28 and ends on page 4, line 4 with the following paragraph.

To certify a Copolymer 1 or terpolymer preparation for use in a pharmaceutical products, it is necessary to accurately determine the molecular weight distribution of the polypeptides in the preparation. One method for determining the molecular weight is chromatography on a Superose 12 column (a cross-linked, agarose-based medium with an exclusion limit of  $2 \times 10^6$  Daltons, an optimal separation range of 1000 to  $3 \times 10^5$  Daltons, and a bead diameter of 20-40  $\mu\text{m}$ ). Calibration coefficients of columns for determination of glatiramer acetate molecular weight have been determined using glatiramer acetate batches with indirectly measured molecular weights. Indirect measures have included viscosimetry and velocity-sedimentation ultracentrifugation. More recently, batches of glatiramer acetate markers have been prepared whose molecular weights were determined by multiple angle laser light scattering (MALLS).

On page 7, replace the paragraph the begins on line 24 and ends on line 27 with the following paragraph:

Figures 1a-1, 1a-2 and 1a-3 provide[s] the distribution of alanine in the molecular markers (TV-markers) described in Table 1. The amino acid position is

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defined by the X-axis. The presence of an alanine is indicated by a vertical bar at the indicated amino acid position.

On page 7, replace the paragraph the begins on line 28 and ends on line 30 with the following paragraph:

Figures 1b-1, 1b-2 and 1b-3 provide[s] the distribution of lysine in the TV-markers described in Table 1. The amino acid position is defined by the X-axis. The presence of a lysine residue is indicated by a vertical bar at the indicated amino acid position.

On page 8, replace the paragraph the begins on line 1 and ends on line 3 with the following paragraph:

Figures 1c-1, 1c-2 and 1c-3 provide[s] the distribution of glutamic acid in the TV-markers described in Table 1. The amino acid position is defined by the X-axis. The presence of a glutamic acid residue is indicated by a vertical bar at the indicated amino acid position.

On page 8, replace the paragraph the begins on line 4 and ends on line 6 with the following paragraph:

Figures 1d-1, 1d-2 and 1d-3 provide[s] the distribution of tyrosine in the TV-markers described in Table 1. The amino acid position is defined by the X-axis. The presence of a tyrosine residue is indicated by a vertical bar at the indicated amino acid position.

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On Page 14, replace the table that begins on line 5 and ends on line 15 with the following table:

TABLE 1 Selected TV-markers amino acid sequences

TV-##	SEQ ID NO	Sequence
<u>TV-35</u>	1	AKKYAKKEKAACKKAYKKEAKAKAAEAAAKEAAYEA
<u>TV-45</u>	2	AKKYAKKAKAEKAKKAYKAAEAKKAAYEKAAAEKAAAKE- AAYEA
<u>TV-56</u>	3	AKKYAKKEKAYAKKAKEKAACKKAEAKAYKAAEAKKKAEAKY- KAEAAKAAAKEAAYEA
<u>TV-66</u>	4	AKKYAKKEKAYAKAKKAEAKAAKKAKAEAKKYAKAAKAEK- KEYAAAEAKYKAEAAKAAAKEAAYEA
<u>TV-77</u>	5	AKKYAKKEKAYAKKAKEKAACKKAEAKAYKAAEAKKKAKAEA- KKYAKAAKAEKKEYAAAEAKYKAEAAKAAAKEAAYEA
<u>TV-86</u>	6	AKKYAKKEKAYAKKAKEKAACKKAEAKAYKAAEAKKKAKAEA- KKYAKAAKAEKKEYAAAEAKYKAEAAKKAYKAEAAKAAAK- EAAYEA
<u>TV-109</u>	7	AKKYAKKAEKAYAKKAKAAKEKKAYAKKEAKAYKAAEAKK- KAKAEAKKYAKEAAKAKKEAYKAEAKKYAKAAKAEKKEYA- AAEAKKAEAAKAYKAEAAKAAAKEAAYEA

On page 23, replace the paragraph the begins on line 32 and ends on line 36 with the following paragraph:

Figures 1a-1, 1a-2, 1a-3, 1b-1, 1b-2, 1b-3, 1c-1, 1c-2, 1c-3, and 1d-1, 1d-2 and 1d-3 provide the distribution of alanine, lysine, glutamic acid and tyrosine, respectively, in the TV-markers described in Table 2. The amino acid position is defined by the X-axis, with the first amino acid corresponding to the C-terminal position. The presence of an

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amino acid is indicated by a vertical bar at the indicated amino acid position.

Page 56, replace the two paragraphs that begin on line 2 and end on line 10 with the following one paragraph:

The present invention provides molecular weight markers for accurate determination of the molecular weight of glatiramer acetate and other copolymers. The present invention further provides a plurality of molecular weight markers for determining the molecular weight of glatiramer acetate and other copolymers which display linear relationships between molar ellipticity and molecular weight, and between retention time and the log of the molecular weight. The molecular weight markers also optimally demonstrate biological activity similar to glatiramer acetate ~~ereerresponding~~ or corresponding copolymers and can be used for treating or preventing various immune diseases. In addition, the subject invention provides pharmaceutical compositions for the treatment of immune diseases comprising a polypeptide having an identified molecular weight and an amino acid composition corresponding to glatiramer acetate or a terpolymer.